



Calcium

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1105

(Version 1.3E)

Detection and Quantification of Calcium(Ca^{2+}) Content in Serum,
Urine, Saliva and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Calcium is essential for all living organisms, where Ca^{2+} sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes. 99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissue. Serum calcium levels are tightly controlled (8.4-11.4 mg/dL) and any variation outside this range can have serious effects. Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. Calcium ion channels control the migration of calcium ions across cell membranes, permitting the activation and inhibition of a wide variety of enzymes. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels that can occur in severe alcoholism. In humans, when the blood plasma ionized calcium level rises above its set point, the thyroid gland releases calcitonin, causing the plasma ionized calcium level to return to normal. When it falls below that set point, the parathyroid glands release parathyroid hormone (PTH), causing the plasma calcium level to rise.

The calcium ions can react with MTB. The products can be measured at a colorimetric readout at 612 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 mlx 1	4 °C
Masking Reagent	Powderx 1	4 °C, keep in dark
Masking Reagent Diluent	1.5 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard (3 mmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 612 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.187/0.093 mmol/L.

Dye Reagent: Add 8 ml distilled water to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2-3 months.

Masking Reagent: Briefly centrifuge prior to opening. Add 1 ml Masking Reagent Diluent to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2-3 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For serum sample

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Blank	Standard	Sample**
Reaction Buffer	100 μ l	100 μ l	100 μ l
Distilled water	10 μ l	--	--
Standard	--	10 μ l	--
Sample	--	--	10 μ l
Masking Reagent	10 μ l	10 μ l	10 μ l
Dye Reagent	80 μ l	80 μ l	80 μ l
Mix, wait for 5 minutes, measure at 612 nm and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

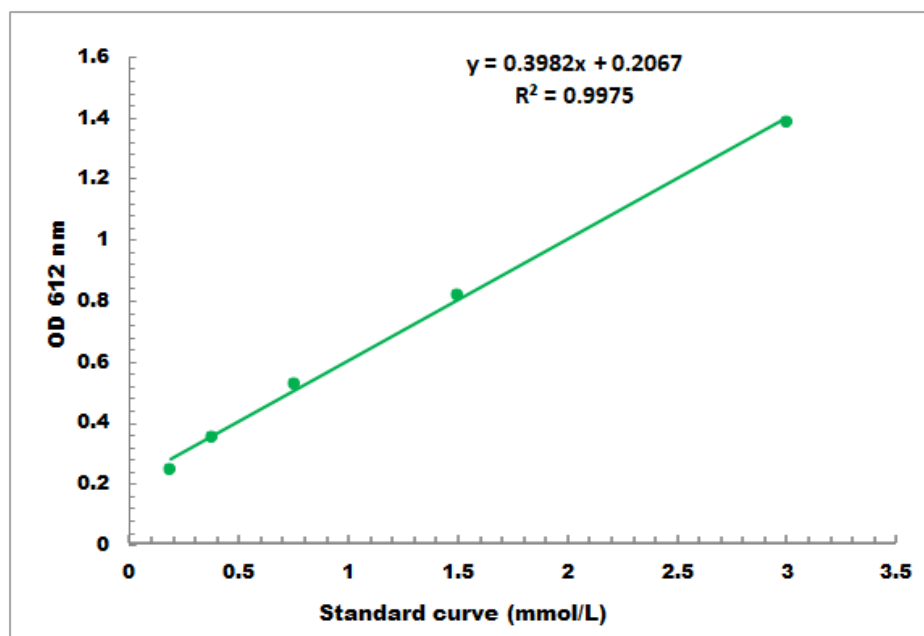
C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1mmol/L -3mmol/L